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# PROCEEDINGS
OF THE
# INDIAN ACADEMY
# OF SCIENCES

VOL. XLI] *SECTION B* [NO. 4

APRIL 1955

*Price Rs. 2 or 3 Sh.* *Annual Subscription Rs. 18*

PRINTED AT THE BANGALORE PRESS, BANGALORE CITY, BY C. VASUDEVA RAO
SUPERINTENDENT AND PUBLISHED BY THE INDIAN ACADEMY OF
SCIENCES, BANGALORE

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# A CONTRIBUTION TO THE FLORAL MORPHOLOGY AND EMBRYOLOGY OF *UTRICULARIA RETICULATA* SMITH

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AND

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Received January 7, 1955

(Communicated by Professor L. Narayana Rao, F.A.Sc.)

The literature on the floral morphology and embryology of members belonging to the family Lentibulariaceæ includes the recent detailed studies by Wylie and Yocom (1923), Kausik (1938) and Khan (1954). The last named author has recently reviewed this literature and it is, therefore, unnecessary to give here again a resume of these and other older investigations relating to this subject.

The plant selected for the present work is *Utricularia reticulata* Smith which is commonly met with in paddy fields in various parts of South India. The material for this investigation was collected near Karwar in the Bombay Province during a recent botanical study tour there. The material was fixed in formalin-acetic-alcohol and passed through the usual processes of dehydration and infiltration. Sections were cut at 10–14 $\mu$ and stained in Heidenhain's iron-alum hæmatoxylin. To study the pollen grains whole mounts in methyl-green glycerine jelly were made according to the method outlined by Wodehouse (1935). Temporary lactic acid preparations were prepared to sketch the pollen grains.

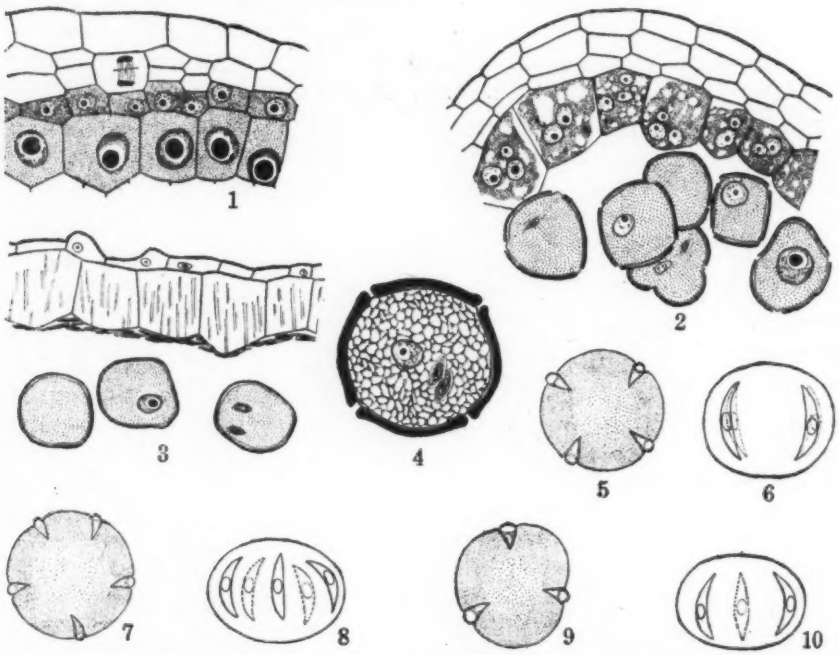
## The Flower

The bluish purple flowers are fairly large and are borne on long twining scapes. The two calyx lobes are much enlarged in the fruit. The zygomorphic corolla has a large lower lip and the spur is slightly curved. The two epipetalous stamens are anterior in position. The antero-posteriorly compressed gynœceum has a hollow style and bowl-shaped stigma. The anterior lip is larger than the posterior one. The central placenta is flattened anteroposteriorly (Fig. 11).

## Microsporogenesis and the Male Gametophyte

A layer of hypodermal archesporial cells is formed in each lobe of the young anther. This layer divides periclinally to form an outer primary

parietal layer and an inner sporogenous layer. The primary parietal layer next divides, in its turn, to give rise to three layers of cells, the endothecium, a single middle layer and the tapetal layer (Figs. 1, 2). During subsequent development the cells of the middle layer get somewhat crushed and the




FIGS. 1–10. *Utricularia reticulata*. Fig. 1. A portion of young anther lobe showing archesporium, tapetal layer and wall, ×647. Fig. 2. A portion of a fairly old anther lobe showing pollen grains, tapetal layer, middle layer, endothecium and epidermis; note the presence of bi- and tri-nucleate tapetal cells, ×647. Fig. 3. A portion of mature anther lobe with persistent endothecium, the dwindling epidermis and pollen grains, ×647. Fig. 4. Sectional view of a tetra-colporate pollen grain showing three-celled condition, ×647. Figs. 5–10. Psilate pollen grains at different views showing the disposition of colpi and pores.

tapetal cells become large and two- or three-nucleate (Fig. 2). In still later stages the endothecium shows the characteristic fibrous thickenings, while the overlying epidermal cells become slightly crushed and crumpled here and there (Fig. 3).

The cells forming the sporogenous layer are in the mean time gradually differentiated to form the microspore mother cells. These undergo the usual reduction divisions and give rise to the tetrads of microspores. The microspores acquire their characteristic rounded form to become pollen grains

with their wall differentiated into a thick exine and a thin intine. The pollen grains are three-celled when they are ready to be shed (Fig. 4).

The fully developed pollen grains measure about 29–31 $\mu$ and are generally tetra-colporate. Tri- and penta-colporate grains are also not uncommonly met with. The tri-colporate grains are relatively slightly smaller in size than the tetra- or penta-colporate ones. The colporate nature of the pollen grains is clearly made out in an equatorial view. The furrows are seen to be short and do not extend to the poles of the grains; the pores are restricted to the centre of these furrows (Figs. 5–10).

### Megasporogenesis and the Female Gametophyte

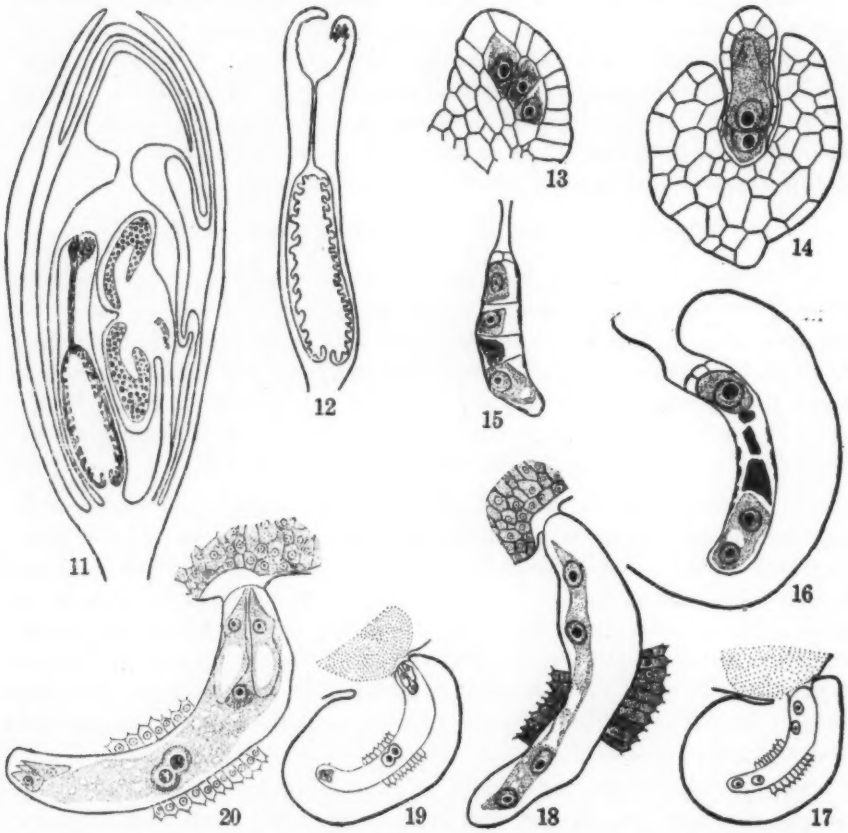
The flattened massive central placenta gives attachment to a very large number of anatropous tenuinucellate ovules all over its surface except at the apex where a clear dome-like summit is left free of ovules (Figs. 11, 12). Opposite each ovule on the placenta is formed a large and richly cytoplasmic mass of cells which constitutes the placental nutritive tissue (Figs. 17–20, 28). This tissue plays a prominent role in the nutritive mechanism of the developing ovule and later of the seed.

In a young ovule the nucellus shows a single hypodermal archesporial cell; occasionally, however, more archesporial cells than one are met with (Figs. 13, 14, 16). The archesporial cell functions directly as the megaspore mother cell which gives rise, after undergoing the reduction division, to a linear tetrad of megaspores (Fig. 15). Invariably it is the lowest megaspore which develops into the female gametophyte, while the upper three megaspores begin to break down rapidly. In Fig. 15 is shown a slight variation which is frequently met with, where the third megaspore of the tetrad is the first to undergo degeneration.

Fig. 16 represents a case where more archesporial cells than one, in this case two such cells, are met with in an ovule. One of these has already developed into a linear tetrad of megaspores, whereas the other one is still undivided.

The functioning lowest megaspore of the linear tetrad elongates rapidly and its nucleus undergoes three successive divisions. After these divisions are over, an eight-nucleate embryo sac is formed according to the Polygonum-type of development. The mature embryo sac is next organized with its usual cell-complements of an egg-apparatus and three antipodal cells, with the two fusing polar nuclei lying somewhere about the centre of the sac (Fig. 20). In shape the embryo sac is prominently curved in the sagittal plane of the ovule and appears more dilated at its micropylar end than it is

at the antipodal end (Figs. 19, 20). Though there is considerable elongation of the embryo sac during the successive stages of its development and growth, the micropylar end of the sac does not project out beyond the limit



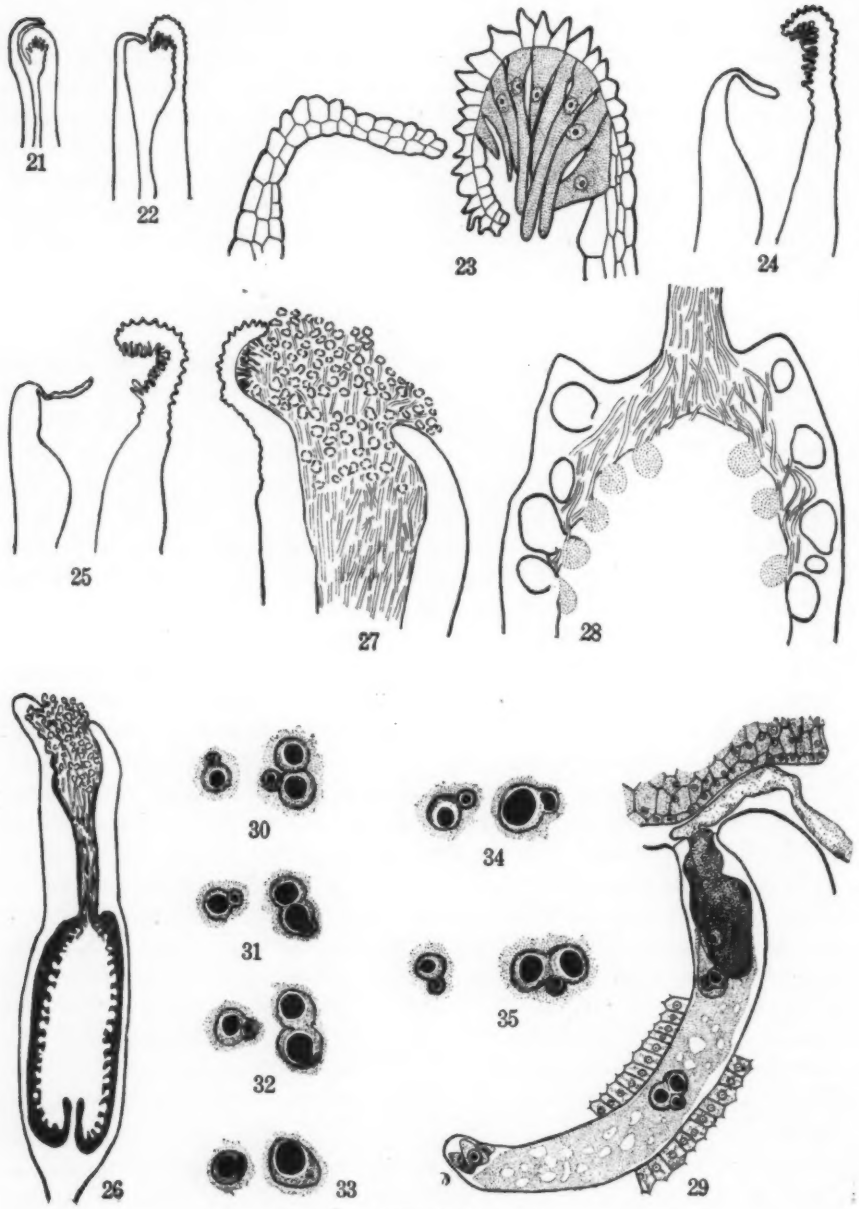

FIGS. 11–20. *Utricularia reticulata*. Fig. 11. L.s. flower bud showing different floral parts, ×25. Fig. 12. L.s. of young gynœceum to show the stigmatic flaps, hollow style and free-central placentæ; note the absence of ovules at the summit of the placentæ, ×50. Fig. 13. Young nucellar primordium with three archesporial cells, ×647. Fig. 14. Same at a later stage showing two archesporial cells, ×647. Fig. 15. A linear tetrad of megaspores with degenerated nucellar cells; note the degenerated third megaspore, ×647. Fig. 16. L.s. of ovule with an archesporial cell and a linear tetrad of megaspores of which the upper three have degenerated, ×647. Fig. 17. Diagrammatic sketch of ovule with four-nucleate embryo sac and placental nutritive tissue, ×215. Fig. 18. Same enlarged with placental nutritive cells and the integumentary tapetum, ×647. Fig. 19. Diagrammatic sketch of ovule at the matue embryo sac stage, ×215. Fig. 20. Mature embryo sac with synergids, egg, secondary nucleus and three antipodal cells; note the space between the micropylar ends of the embryo sac and the nutritive tissue, ×647.

of the integument (Figs. 17–20) and does not, therefore, appear to become extra-ovular as in the other species investigated earlier where this feature is conspicuously seen. The micropylar end of the sac, however, reaches to the opening of the ovule and the apical part of the egg-apparatus lies exposed here, but placed almost in contact with the placental nutritive tissue opposite. A micropyle, in the real sense of the term, cannot, therefore, be regarded as being organised in this species too as in the other investigated forms of *Utricularia*. A conspicuous integumentary tapetum develops in the middle portion of the embryo sac (Figs. 17–20).

## Pollination and Fertilization

Pollination in *Utricularia reticulata* is autogamous. The flattened gynœceum has a short hollow style and a deep bowl-like stigma which is formed of two distinct flaps. One of these flaps is slightly larger than the other. At the time of the differentiation of the archesporium in the young ovule, the larger flap of the stigmatic bowl is strongly incurved and is also partially hooded over by the smaller flap (Fig. 21). On the inside of the incurved larger flap are seen prominent elongated cells which grow in the form of hairs and are secretory in function (Figs. 22, 23). As the gynœceum develops further, when the linear tetrad of megaspores is seen in the ovule, the distal incurved region of the larger flap begins to straighten up somewhat and so slips out of the hood-like smaller flap (Figs. 22–25), by which time the ovules contain fertilizable embryo sacs. The anthers, which are situated on the anterior side of the flower, now face and are also brought into close approximation with the exposed inside of the larger stigmatic flap. The pollen from the dehisced anthers is now shed on the exposed inside of the larger flap where they are caught and retained by the secretions of the hairs. The two stigmatic flaps are doubtless of help in effectively holding together between them, as in a deep bowl, hundreds of pollen grains which are shed from the anthers (Figs. 26, 27). Being thus provided with an extremely favourable situation, the pollen grains germinate in the stigmatic bowl and send out down through the hollow style their growing tubes in large numbers (Figs. 26, 27). These "bundles" of pollen tubes pass through the hollow style and enter the ovary cavity where they reach the dome-like summit of the placenta (Figs. 26, 28). They continue their growth further here creeping all over the surface of the placenta in between the ovules (Fig. 28).

After traversing some distance on the placenta the pollen tube passes round the funiculus of the ovule and approaches the micropylar end of the embryo sac from a side by squeezing itself through the narrow space left




FIGS. 21–35

FIGS. 21–35. *Utricularia reticulata*. Figs. 21–22. L.s. of stigma and style with two stigmatic flaps, ×50. Fig. 23. Enlarged view of Fig. 22 to show two-layered apices of stigmatic lobes, secretory cells and conical cells, ×129. Figs. 24–25. L.s. of stigma and style to show the behaviour of the stigmatic flaps at different stages of development, ×50. Fig. 26. L.s. of gynœceum with pollen grains in the stigmatic cup, pollen tubes in the hollow style and subsequently reaching the placental summit, ×30. Fig. 27. Enlarged sketch of the stigmatic cup with large number of pollen grains and germinating pollen tubes in the style, ×50. Fig. 28. Enlarged view showing the pollen tubes alighting directly on the placental summit and their further movement between the ovules, ×129. Fig. 29. A stage in double fertilization and also showing the course of pollen tube between the placental nutritive tissue and the micropylar end of the ovule, ×647. Figs. 30–35. Various stages showing syngamy and triple fusion, ×970.

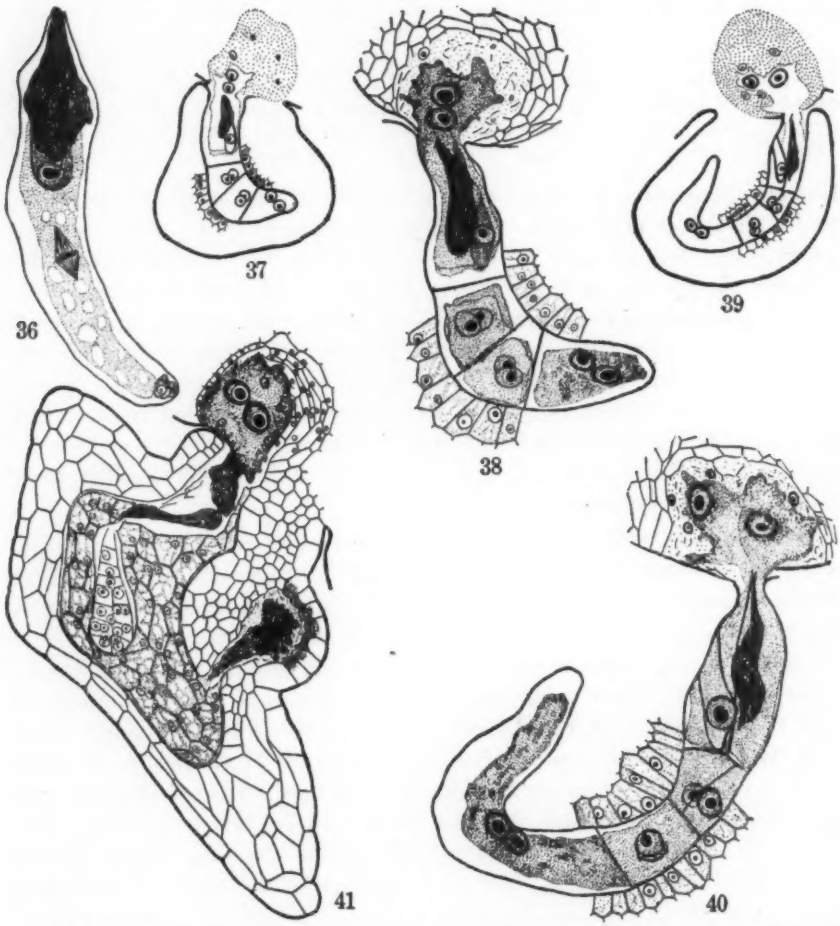
between the ovule and the placenta (Fig. 29). It then comes in direct contact with the exposed apical region of the egg-apparatus and enters the embryo sac through and by destroying the nearer synergid. The other synergid escapes this impact of the entering pollen tube and so remains intact for quite some period subsequently. The persisting synergid can be made out very much after fertilization is over and when the initial endosperm cells and the haustoria are already fully differentiated. After the contents of the pollen tube are discharged into the embryo sac, the first male nucleus fuses with the egg and the second travels further down in the sac and participates in triple fusion (Fig. 29). In this process of double fertilization, triple fusion precedes syngamy.

Stages in syngamy and triple fusion were observed in several preparations and in every one of them it was found that the male nuclei are more deeply stained than the other nuclei participating in double fertilization. It is also interesting to note here that triple fusion takes place in one of three somewhat distinct ways as reported earlier in the case of *Utricularia cærulea* (Kausik, 1938). These three ways are: (*a*) the second male nucleus fusing with the secondary nucleus formed by the fusion of the two polar nuclei (Fig. 34); (*b*) the male nucleus fusing first with one of the two polar nuclei which have merely come together but have not as yet fused (Fig. 30) and (*c*) the male nucleus and the two polar nuclei fusing simultaneously (Fig. 35). The successive stages in triple fusion according to the second method and corresponding stages in syngamy were followed in some detail and are shown in Figs. 30–33.

## ENDOSPERM

The endosperm is *ab initio* cellular and follows the mode of development according to the Scutellaria-type as in the other species of *Utricularia*. The first division of the primary endosperm nucleus (Fig. 36) results in the formation of two chambers. The next division is by vertical walls in both the chambers and these are incomplete at the two ends of the sac. By

further divisions the 8-celled stage is reached in which the two central tiers of cells are the endosperm cells, while the two end tiers are next organised as the micropylar and chalazal endosperm haustoria (Figs. 37–40). At




FIGS. 36–41. *Utricularia reticulata*. Fig. 36. A stage showing first division of primary endosperm nucleus, ×647. Fig. 37. Diagrammatic sketch of the ovule showing eight-celled stage of endosperm with micropylar and chalazal haustoria, ×574. Fig. 38. Enlarged view of the same; note the supernumerary nuclei in the micropylar haustorium, ×647. Fig. 39. Diagrammatic sketch of ovule showing eight-celled endosperm at a later stage, ×574. Fig. 40. Same enlarged ; note the extension of chalazal haustorium and the presence of supernumerary nuclei in the micropylar haustorium, ×647. Fig. 41. L.s. of a fairly old seed showing the integument, endosperm and embryo ; note the supernumerary nuclei in the micropylar endosperm haustorium and the degenerated chalazal endosperm haustorium, ×574.

first the chalazal haustorium is more aggressive than the micropylar one and it rapidly destroys the chalazal tissue of the ovule and penetrates deep down to the epidermis of the seed, whereupon its further growth is stopped (Figs. 39–41). Its activity thus comes to an end and it persists in subsequent development of the seed as a darkly-staining mass pressed against the epidermis (Fig. 41). Here, the epidermis develops lamellations on its inner walls in contact with the tip of the chalazal haustorium (Fig. 41). The micropylar haustorium, which at first is less conspicuous, is extra-ovular and becomes more and more aggressive as the activity of the chalazal haustorium begins to decline. It appears as a conspicuous bulbous structure and with its two prominent haustorial nuclei, penetrates rapidly into the extensive placental nutritive tissue (Figs. 37–41). The cells of the latter tissue begin to break down on coming into direct contact with the haustorium and their cytoplasm and nuclei form a common viscid substance within which the haustorium lies (Figs. 37–41). The whole of the nutritive tissue is thus gradually, but rapidly, used up by the haustorium during the development of seed and a large depression is formed in its place on the surface of the placenta (Figs. 37–41). A similar feature has been noted in *Utricularia vulgaris americana* (Wylie and Yocom, 1923).

## Discussion

The account presented here of *Utricularia reticulata* conforms in general to the earlier accounts of some of the other investigated species. The pollen grains are distinctly three-celled at the time of shedding as in the case of *Utricularia cærulea*. In *U. flexuosa*, on the other hand, Khan (1954) reports that the pollen grains are three-nucleate. Further, the several abnormalities which he has figured and described were also not met with in the present species.

The number of furrows exhibited by the pollen grains of *Utricularia reticulata* seems to be variable. This number may be three, four or five. According to Erdtman (1943) there are as many as fifteen to twenty furrows in *U. intermedia*, *U. minor* and *U. neglecta*. In *U. vulgaris americana* (Wylie and Yocom, 1923) and *U. flexuosa* (Khan, 1954) several furrows seem to be present, while in *U. cærulea* the figured pollen grains, although not described, are seen to have three and four furrows (Kausik, 1938).

Recently, in a master key for the pollen grains of some angiosperms Fægri and Iversen (1950) have given the many pored (stephanocolporate) condition as being characteristic of the genus *Utricularia*. However, in view of what has been said above the number of pores in the pollen grains

seem to be variable as noted not only in *U. reticulata* but also in some of the other species. Therefore, the number of pores may not be a reliable criterion in taxonomic determination of the species of *Utricularia.*

The central placenta is much flattened in *Utricularia reticulata*, whereas in *U. cærulea* and *U. flexuosa* it is distinctly globular. Ovules are borne on all over the surface of the placenta in *Utricularia vulgaris americana* and *U. flexuosa* while in *Utricularia reticulata* the apex of the placenta is devoid of any ovules (Figs. 11, 12, 28) as in *U. cærulea* (Kausik, 1938) and some species of *Genlisea* (Merl, 1915). The ovules do not have any vasculature as in other species.

Two- and three-celled archesporial tissues are met with in the ovules of *Utricularia reticulata*, as also in *U. flexuosa* (Khan, 1954). In the latter species some variations of megaspore tetrads have also been recorded. In *U. reticulata*, although these variations were not all seen, in one case of a two-celled archesporium, one had already developed into a tetrad while the other was undivided (Fig. 16).

Several embryo sacs are seen to develop up to various stages in the same ovule in *Utricularia flexuosa* (Khan, 1954). In the present case, however, a single embryo sac was invariably formed in all the preparations, though two- and three-celled archesporia were seen in earlier stages.

*Utricularia reticulata* does not appear to show any appreciable extra-ovular development of embryo sac which is a common feature in the other species. The embryo sac, on the other hand, just reaches to the surface of the ovule where the apex of the egg-apparatus becomes somewhat exposed but lies close to the placental nutritive tissue. Here it comes in direct contact with an approaching pollen tube which enters, as already described earlier, by squeezing itself through the narrow passage between the ovule and the placenta.

The flowers of *Utricularia reticulata* are well adapted to self-pollination. Cleistogamy seems to be predominant in many species of this genus (Kerner, 1891 and Knuth, 1908). The structure and development of the stigmatic bowl in *U. reticulata* appears to be interesting in so far as they seem to aid an autogamous mode of pollination. The movements of the stigmatic flaps, the close correspondence in the developmental stages of the male and the female gametophytes and anterior disposition of the stamens which come in direct contact with the inside of the larger stigmatic flap seem to be structural adaptations for this mode of pollination in *U. reticulata.* Further, the presence of thick " bundles " of pollen tubes in the hollow style seems to indicate a great viability of pollen and the consequent maximum

seed-setting. Autogamy seems to be the general case where such thick masses of pollen tubes enter the style. Pollination in *U. reticulata* occurs at the time of anthesis and when the flowers are subsequently in full bloom the pollen tubes are already half-way in the style.

The pollen tubes reach the ovules after first alighting on the apex of the placental column. They subsequently grow on the surface of the placenta by creeping in between the ovules. In *U. flexuosa* some of the pollen tubes creep on the surface of the placenta while others grow along the wall of the ovary (Khan, 1954).

Triple fusion may occur in one of three ways as described in the paper. The different ways are noticed in different ovules of one and the same ovary. This is perhaps to be correlated with the time of discharge of the male nuclei into the embryo sacs. If the male nuclei enter an embryo sac sufficiently early, that is, when the polar nuclei have not fused as yet, the second male nucleus fuses with one of the polar nuclei. If the male nuclei are discharged later, the male nucleus fuses with the secondary nucleus which has already been formed. In other cases where the liberation of the male nuclei and the fusion of the polar nuclei are synchronous, all the three fuse together simultaneously. In all cases, however, triple fusion precedes syngamy.

The endosperm develops as in the other investigated species. The micropylar and chalazal haustoria are each two-nucleate and are at the height of their activity in two different periods. The micropylar haustorium is large and bulbous. The chalazal haustorium is narrow, prominently elongated with its lower end growing fast through the chalazal tissue and subsequently reaching the epidermis of the seed where it spreads out as a disc (Fig. 41). In *Utricularia flexuosa* an occasional case of a three-nucleated chalazal haustorium has been reported (Khan, 1954). No such variation was met with in the present case.

## Summary

The paper deals with certain aspects of floral morphology and embryology of *Utricularia reticulata*. The pollen grains are three-celled at the time of shedding. They are psilate and tri-, tetra- or penta-colporate.

The ovary is flattened and bi-carpellary with a large number of anatropous unitegmic ovules borne on a flattened central placenta. The ovules develop all over the surface of placenta except at its dome-like summit.

Megasporogenesis and the development of female gametophyte are recorded. The embryo sac does not grow beyond the integument, but just

reaches to the surface of the ovule where it comes in contact with a placental nutritive tissue.

Pollination is autogamous. Double fertilization occurs regularly, triple fusion always preceding syngamy.

Endosperm is *ab initio* cellular and follows the Scutellaria type of development. The micropylar and chalazal haustoria are binucleate and differentiated almost simultaneously. The micropylar haustorium is less prominent in initial stages becoming more and more aggressive only later as the chalazal haustorium begins to decline. The nuclei of both the micropylar and chalazal haustoria are large, conspicuous and deeply stained.

## ACKNOWLEDGMENT

Our thanks are due to Systematic Botanist, Coimbatore, for kindly determining the species investigated now.

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# CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

## II. The Stem of *Eclipta alba* Hassk.

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Received November 15, 1954

(Communicated by Dr. T. S. Sabnis, F.A.SC.)

### Introduction

*Eclipta alba* Hassk. (*Elcipta erecta* Linn.), a member of the family Compositæ, is the only species of the genus *Eclipta* Linn. occurring in our country. It occurs as a common weed in pasture lands, along roadsides and on the margins of lakes and streams. It is found to grow abundantly in nitrogenous soils. Sabnis (1921) has described the species from the Indian Desert. The plants flower throughout the year.

The stem of *Eclipta alba* Hassk. is erect or prostrate, usually much branched and frequently rooting at the nodes. The surface of the stem is strigose with appressed white hairs.

### Previous Work

As far as the author is aware, but for the brief account of the physiological anatomy of the axis and leaf of this species (based on the studies of dried herbarium specimens) given by Sabnis (1921, 1929), the present contribution by the author is the first detailed report of the various features of the physiological anatomy of its stem. Besides, Sabnis has described the anatomical characters of the xerophytic form of this species while the description given below refers to its hydrophytic amphibious form.

### Material and Method

Specimens of *Eclipta alba* Hassk. have been collected from different habitats in and around Nagpur. The species has been found to grow at various spots of varying moisture content. It has also been specially grown in artificial swamps in the Botanical Garden of the Government College of Science, Nagpur.

Anatomical studies presented in this paper are based on hand-cut sections of fresh and preserved material. The fixative used was Formalin Acetic

Alcohol. The microtome method was found to be particularly unsuitable while dealing with the soft submerged parts of the stem.

## DESCRIPTION

It will be convenient to describe the anatomical characters of the aerial and submerged parts of the stem separately in order to emphasise the amphibious nature of the species.

### A. *Aerial Part*

(i) *Epidermis.*—A single layer of rather thick-walled cells. Cuticle thin or entirely absent. A number of glandular multicellular trichomes with thick and tuberculate walls (Fig. 4). Anthocyanin develops in the epidermal cells on the onset of drier and warmer conditions. It is found in all specimens collected from comparatively drier soils and may extend right upto the inner cortex.

The stomata deserve a special mention on account of their peculiar disposition. They are not distributed uniformly over the general epidermis but are confined to special areas where they occur in groups. These "stomatal islands" are raised above the general level of the epidermis and comprise cells that are thin-walled, chlorophyllose and isodiametric. These cells, thus, differ markedly from the ordinary epidermal cells which are rectangular in outline and thick-walled (Figs. 1 and 2). Considerable difficulty is experienced in obtaining the stomata in a transverse section of the stem (Fig. 3) on account of the raised position and delicate nature of the entire stomata-containing area.

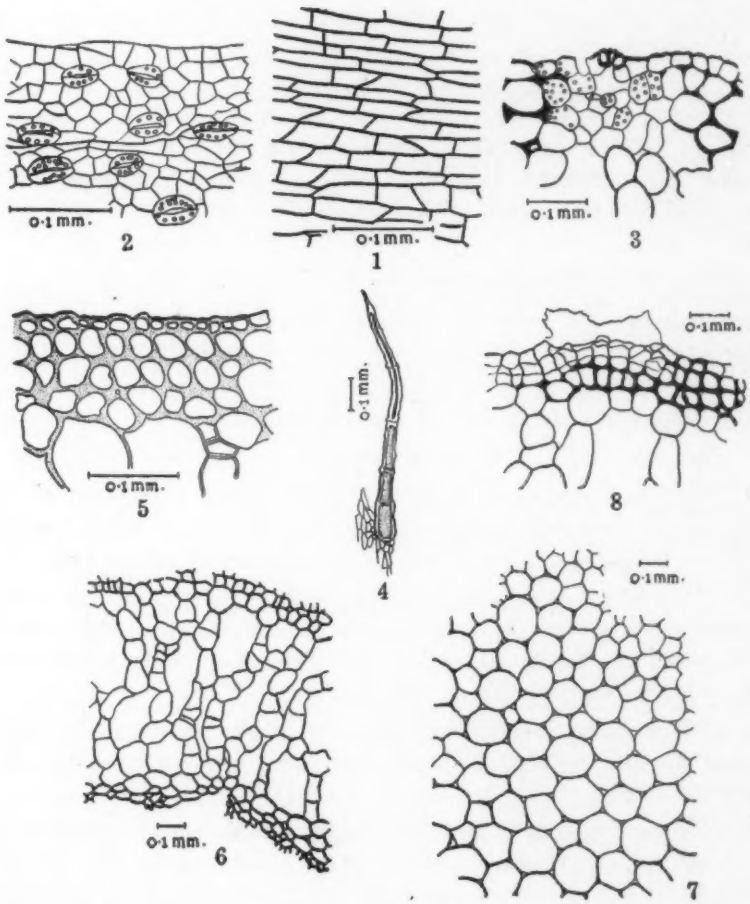
(ii) *Cortex.*—The outer cortex consists of two to three layers of well-developed collenchyma (Fig. 5). But, the continuity of collenchyma is interrupted in regions situated below the stomata. Here the cells are thin-walled and chlorenchymatous (Fig. 3).

The middle cortex is lacunar. The lacunæ are small and are bounded by round cells containing chloroplasts. The stem is found to retain this feature permanently, for, it figures even in the specimens collected from very dry localities.

The inner cortex is a narrow region of two to three layers of thin-walled parenchyma. Resin-ducts are placed at regular intervals in this region. The cells of the endodermis show prominent starch-grains.

(iii) *Pericycle.*—Heterogeneous; radially alternating zones of thin-walled parenchyma and sclerenchymatous fibres, the latter forming crescent-shaped stereid bundles on the outer periphery of the phloem groups of the vascular bundles.

(iv) *Vascular System.*—This is of the *Helianthus annuus* type. There is a single ring of open, collateral vascular bundles separated by wide medullary rays.




TEXT-FIGS. 1–8. *Eclipta alba* Hassk. Fig. 1. Surface view of the ordinary epidermal cells. Fig. 2. Portion of a "stomatal island" in surface view. Fig. 3. Part of the T.S. of the aerial portion of the stem showing the stoma and the chlorenchymatous patch in the outer cortex. Fig. 4. A trichome. Fig. 5. Part of the T.S. of the aerial portion of the stem showing the collenchyma of the outer cortex. Fig. 6. Part of the T.S. of the submerged part of the stem showing the well-developed lacunar middle cortex. Fig. 7. Part of the T.S. of the submerged portion of the stem showing the pith cells. Fig. 8. Part of the T.S. of the submerged portion of the stem showing the lenticel.

(v) *Pith.*—This is very extensive and consists of roundish, thin-walled cells enclosing small intercellular spaces.

B. *Submerged Part*

(i) *Epidermis.*—Thin-walled; interrupted at places owing to the formation of lenticels (Fig. 8).

(ii) *Cortex.*—The collenchyma of the outer cortex is poorly developed (Fig. 8). The middle cortex is the most conspicuous part of the stem. It consists of definite schizogenous air-spaces that are bounded by radially elongated thin-walled cells (Fig. 6). The inner cortex is similar to the one in the aerial part of the stem. The endodermis contains small-sized starch-grains.

(iii) *Pericycle.*—Simulates the one in the aerial part.

(iv) *Vascular System.*—The submerged part shows the formation of interfascicular strips of cambium and poor secondary growth. Phloem abounds in starch.

(v) *Pith.*—Thin-walled and parenchymatous (Fig. 7).

## DISCUSSION

1. The usual habitats in which *Eclipta alba* Hassk. is reported to occur hardly give any idea of the hydrophytic nature of this species which is brought out in the foregoing account of physiological anatomy of its stem.

2. The absence of a cuticle, the raised position of stomata, the presence of groups of thin-walled chlorenchymatous cells interspersed in the collenchymatous cortex and the lacunar and assimilatory nature of the middle cortex clearly indicate that the stem is well adapted to a marshy habitat.

3. The anatomical differences observed in the corresponding regions of the aerial and submerged parts of the stem is quite characteristic of its amphibious nature.

It is interesting to note that in the transverse section of the submerged part of the stem the lacunar cortex is almost as extensive as the pith, thus bringing about a more central disposition of the mechanical and conducting elements which is so characteristic a feature of the stems of water plants. It shows how fully the submerged part of the stem is adapted to the watery environment and stands in strong contrast to the aerial part which has a peripheral disposition of the mechanical and conducting elements on account of a proportionately extensive pith and a much narrower cortex.

4. It will be useful here to compare the observations of the author about the physiological anatomy of the stem of the hydrophytic form of

*Eclipta alba* Hassk. with those reported by Sabnis (1919, 1929) for the xerophytic form of the species occurring in the Indian Desert. The comparison throws important light on the plasticity of structure which is so characteristic a feature of the helophytes.

| Anatomical Feature | Hydrophytic Form | Xerophytic Form |
|---|---|---|
| Epidermis .. | Epidermal cell of two types:— (i) rectangular and thick-walled (ii) isodiametric, thin-walled and chlorenchymatous | Epidermal cells all similar and thick-walled. |
| | Stomata raised | Stomata depressed |
| Cortex .. | Thin-walled chlorenchymatous cellls interspersed in the collenchyma of the outer cortex | Outer cortex entirely collenchymatous |
| | Middle cortex lacunar and assimilatory, lacunæ well-developed in the submerged part | Middle cortex only assimilatory |
| | Resinducts in the inner cortex | Not recorded |
| | Endodermis contains starch | Not recorded |
| Pericycle .. | Heterogeneous; thin-walled cells alternating radially with stereid bundles | Composite ring of groups of stone-cells |
| Phoelm .. | Abundance of Starch | Not recorded |
| Wood .. | Separate vascular bundles with wide medullary rays; poor secondary growth | Composite |

The thick-walled epidermis, the depressed stomata, the assimilatory cortex which is not lacunar, and a strong development of the mechanical and conducting systems in the form of composite pericycle and wood observed in the stem of the xerophytic form, thus, stand in strong contrast to the corresponding anatomical features described by the author.

5. The pronounced development of anthocyanin in the epidermis and cortex of the stem on the advent of drier and warmer conditions lends further support to the author's view about the role of this pigment, expressed in his earlier contribution (Mirashi, 1954), that it offers protection to the plant against too strong light and temperature.

6. The foregoing account brings out the hydrophytic nature of a member that belongs to the family Compositæ. The phylogenetic significance of these findings has been referred to in the author's work on the physiological

anatomy of the stem of *Cæsulia axillaris* Roxb. and need not be repeated here.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. T. S. Sabnis, D.Sc., F.A.Sc., I.A.S., for his kind interest in the work, valuable suggestions and helpful criticism.

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# RESPONSES OF THE CONTRACTILE MECHANISM OF UNSTRIATED MUSCLE

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Received February 3, 1955

Unstriated muscle which has been killed by heat, is a suitable preparation for the study of certain effects of substances on the contractile mechanism (Singh, 1954 *a*, *b*, *c*; 1955 *b*). Thus substances which denature proteins, cause it to lengthen actively; certain substances which coagulate proteins, cause it to contract. Of particular interest is the contraction produced by strong hydrochloric acid; it simulates the contraction produced through the excitatory mechanism (Singh, 1955 *a*). Heat also produces contraction. In the present research the properties of this heat induced contraction have been investigated.

## Experimental

The experimental procedure has been described previously (Singh and Singh, 1954 *b*, *c*). These experiments were performed on strips of circular muscle from the stomach of the frog, *Rana tigrina*, and from dog's stomach. The muscles were killed by heating to 50° C. for 10 minutes. To produce the contraction by heat, the heat killed muscles were suspended in air at room temperature (18–20° C.), and then the contraction was produced by immersion in saline of the required temperature.

## Results

*Effect of heat.*—The effect of heat depends upon the temperature to which the muscle had been previously treated. If the unloaded muscle is killed by heating to 50° C., and then restored to 20° C., heating it again to 50–60° C. under loaded conditions, causes it to relax only (Fig. 1). Unloaded muscle previously killed by heating to 50° C. relaxes actively if heated to 60° C. (Singh and Singh, 1954 *a*, *b*, *c*). Active relaxation is best demonstrated on frog's stomach muscle; it is abolished if the muscle is heated to 65–70° C.

If heated to 65–70° C., the muscle behaves quite differently. It shows a reversible contraction with increase of temperature (Fig. 2). In these experiments the muscle was initially kept at 20° C., after being killed by heating to 70° C. The temperature was then raised to 40° C. and higher

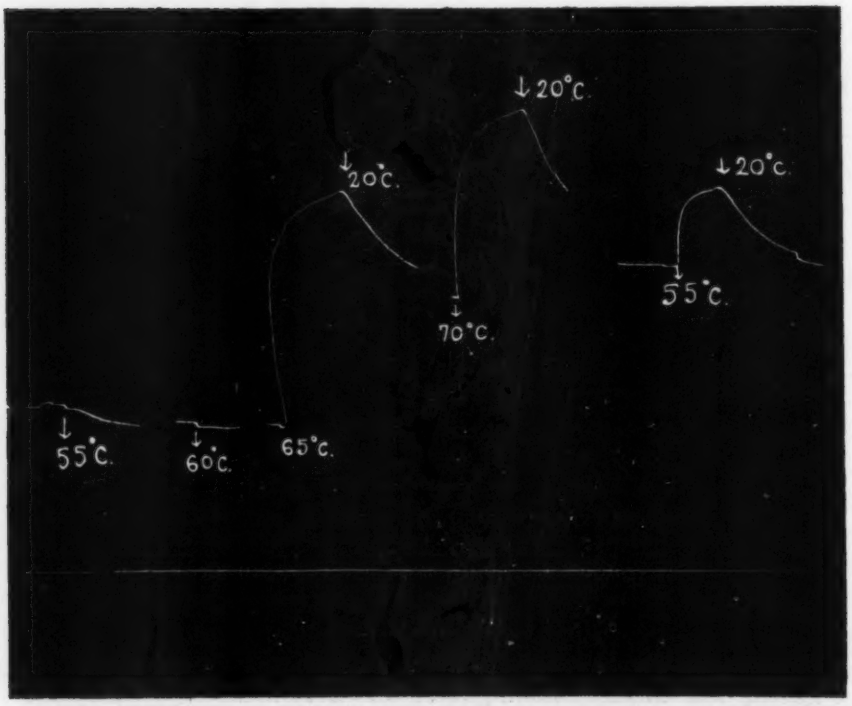


**FIG. 1. Dog's stomach muscle killed by heating to 50° C. and then suspended at 20° C. after each response the muscle is brought back to 20° C. At first 55° and 60° C. cause relaxation only. After the muscle is heated to 70° C., they cause contraction.**

values up to 98° C. These values were increased in steps of 5° C. from 40° C. onwards. The tension varies linearly with temperature up to 80° C.

For further study, the thermal contraction was produced by raising the temperature of the heat-killed muscle to 70° C. each time, the initial temperature being 20° C.

*Sensitivity of different parts of the stomach.*—The thermal contraction of heat killed dog's stomach muscle is maximum at the cardiac end; it diminishes towards the pyloric end, though at this end also, the response may increase. These effects resemble those produced by hydrochloric acid (Singh and Singh, 1955 *a*). This shows that the contractile mechanism of unstriated muscle is a variable entity. The muscle from the cardiac end, though it contracts powerfully relaxes comparatively slowly, when compared with that from other parts of the stomach. Striated muscle from dog's rectus abdominis also contracts powerfully and relaxes very slowly. This

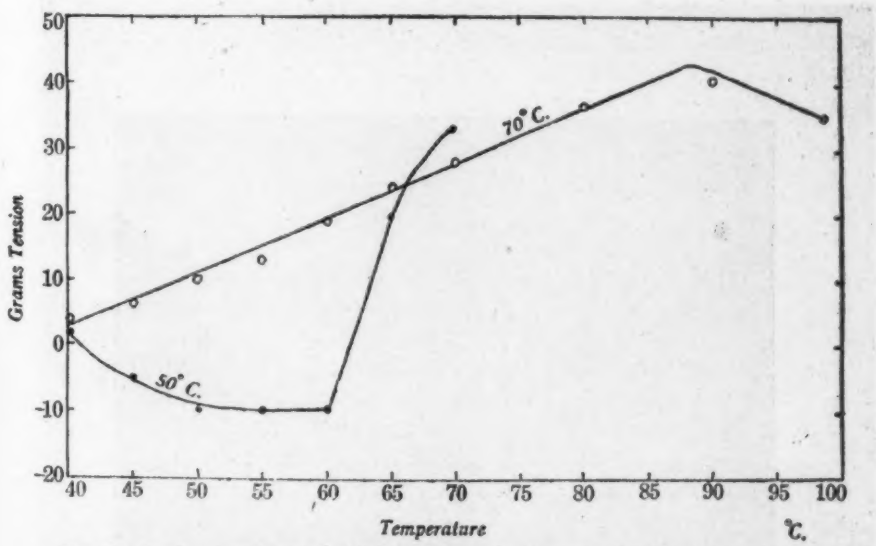


FIG. 2. Dog's stomach muscle killed by heat. Upper curve produced by varying temperatures in muscle at first heated to 70° C. Lower curve by muscle killed by heating to 50° C.

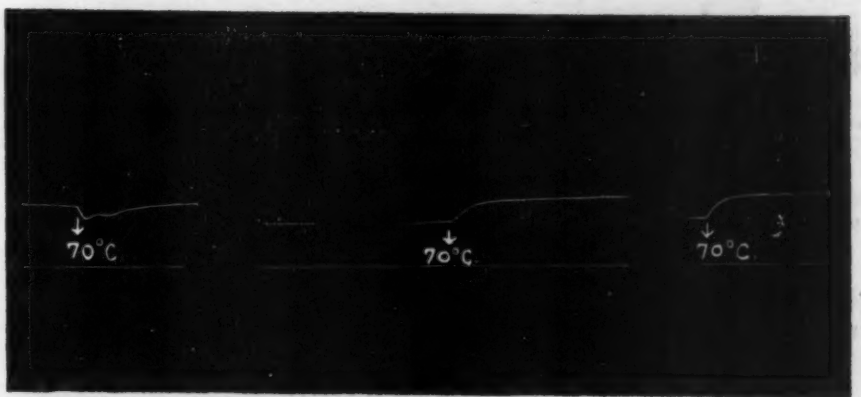


FIG. 3. Dog's stomach muscle killed by heating to 50° C. The second and third responses show the beneficial effect of contraction. The initial dip in the first response is a buoyancy effect, due to immersion of the muscle from air into heated saline.

suggests that the normal response of the contractile mechanism of unstriated muscle is the net result of two opposing forces, which may operate simultaneously; one produces contraction and the other relaxation. The contraction is more powerful if the relaxation is slow, that is, if it is not partially

antagonised by simultaneous rapid relaxation. The muscle may, therefore, be contracting and relaxing simultaneously. This explains the difference between the sensitivity of muscle from various parts of the stomach.

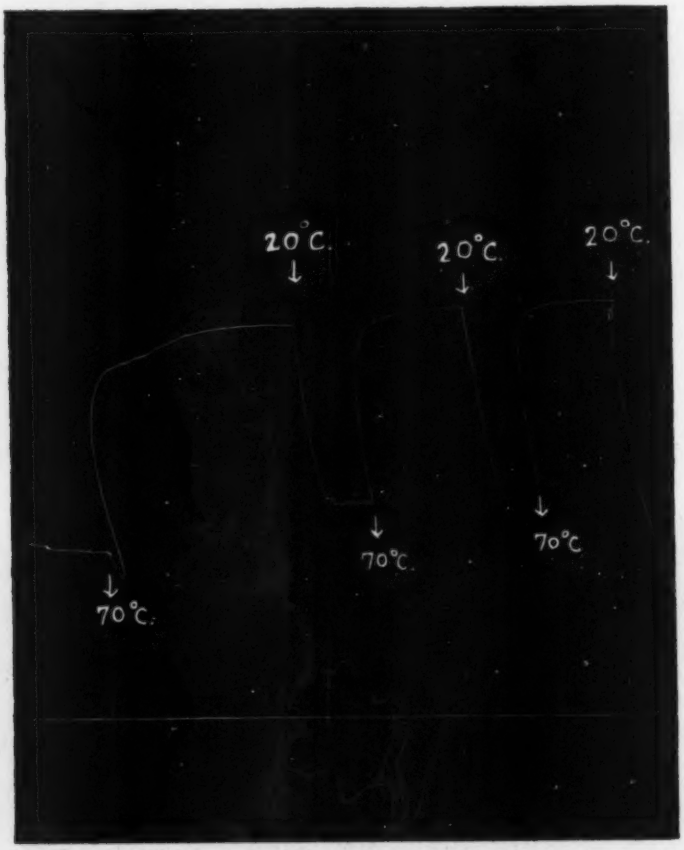


FIG. 4. Dog's stomach muscle from the cardiac end, killed by heating to 50° C. shows fatigue.

*Staircase.*—The staircase phenomenon or the beneficial effect of contraction is shown by the thermal contraction of dog's stomach muscle (Fig. 3). This effect, shown by living muscle, therefore appears to be a property of the contractile mechanism. This is further suggested by the fact that the staircase effect in dead muscle is most marked when the initial response is small, as in muscle from middle part of the stomach or from near the pyloric end. This exactly resembles the staircase effect in living muscle.

FIG. 5*a*. Dog's stomach muscle killed by heating to 50° C.; shows the effect of initial length on tension.

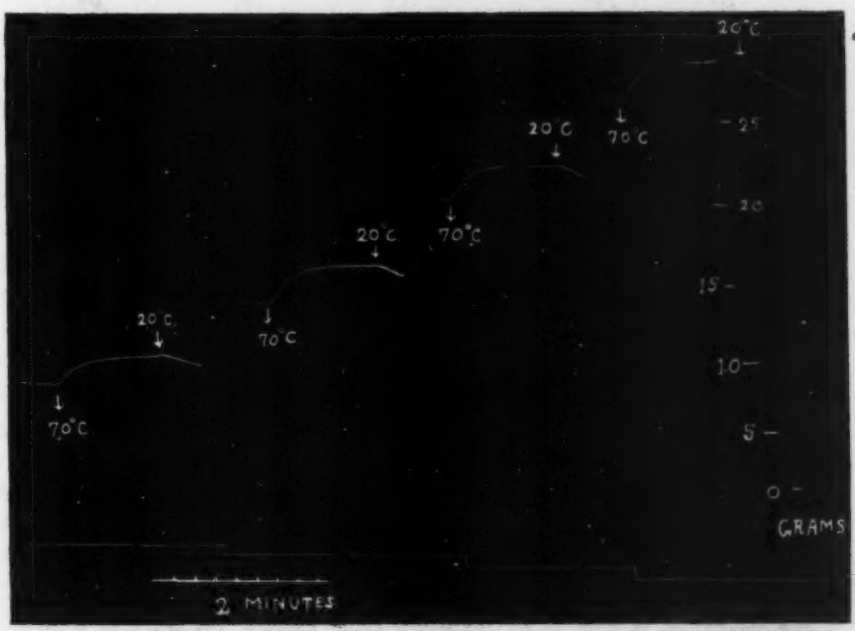


FIG. 5*b*. Frog's heart killed by heating to 50° C. (*Rana tigrina*). Suspended as an ordinary piece of unstriated muscle. Effect of initial length on tension. Length increased by 3–4 per cent each time. Note last contraction is double the first.

In living muscle the staircase effect is most marked if the initial response to alternating current is small. If the response is initially large, then by a little excess of potassium in the saline, or other depressant ions, the initial response of the living muscle can be depressed; the staircase effect is then very pronounced.

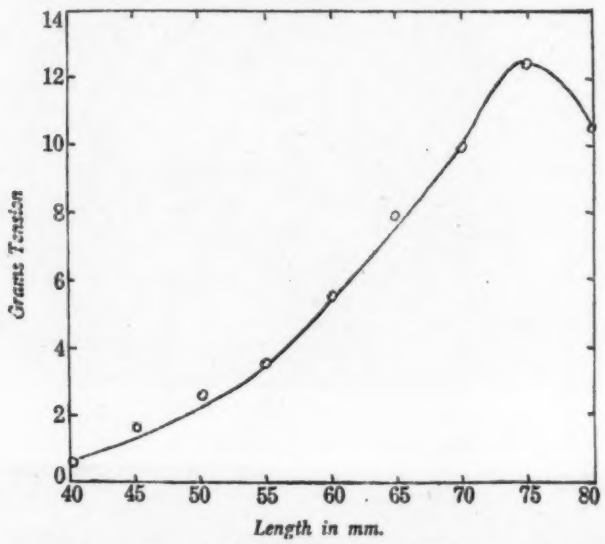


FIG. 6. Dog's stomach muscle killed by heating to 50° C.; shows the effect of initial length on tension.

*Fatigue.*—If the heat killed dog's stomach is made to contract frequently, then " fatigue " sets in. Relaxation becomes incomplete, so that the muscle passes into a contracted state (Fig. 4). This occurs especially in muscle from the cardiac end, in which the thermal response is large. These phenomena simulate those found in living muscle. In living muscle it is found that if the initial contraction is large, then fatigue sets in rapidly, and relaxation may be incomplete. Some changes in fatigue which are shown by living muscle, are therefore due to the contractile mechanism.

*Effect of initial length.*—The thermal response of dead muscle increases with the initial length up to a certain point (Fig. 5 *a*, *b*) therefore, there is an optimum length for contraction (Fig. 6). These effects, therefore, resemble those produced by hydrochloric acid (Singh and Singh, 1955 *a*). This property, found in living muscle, therefore, resides in the contractile mechanism.

It was not possible to demonstrate this property in striated muscle, as the relaxation is very slow. In dog's heart muscle, the thermal contraction

was feeble owing to non-linear arrangement of fibres in the pieces of muscle used. The effect of initial length is best demonstrated with pieces of muscle obtained by transverse sections of the right ventricle. The whole of the frog's heart can be used and suspended like a strip of unstriated muscle (Fig. 5 *b*). In striated muscle, the heat contraction is not reversible as indicated by the

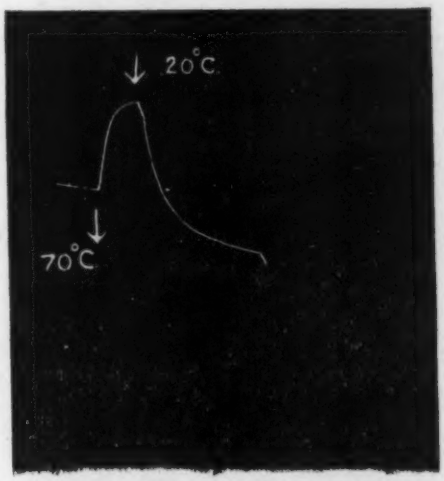


FIG. 7. Dog's stomach muscle killed by heating to 50° C.; shows the relaxation of the thermal contraction beyond the starting point.

slow relaxation, and in cardiac and some unstriated muscles it is partially reversible; this results in a partial tonic contraction, on which reversible contractions can be superimposed, and thus the responses of the contractile mechanism studied. Starling's law of the heart is well demonstrated in this way. It was also demonstrated by contraction produced by hydrochloric acid (Singh and Singh, 1955 *a*).

*Antagonism to tonus.*—The thermal contraction of dead muscle appears to be antagonistic to tonic contraction. In living muscle, tone decreases after a response to alternating current (Singh, 1938 *a*, *b*). If initially tone is great, then the response to alternating current is small, but the subsequent relaxation is more marked. These effects have been ascribed to decrease in viscosity (Winton, 1937). These results are also shown by dead muscle. The muscle continues to relax beyond the starting point after a thermal contraction (Fig. 7). If the initial response is small due to greater tone, then the subsequent relaxation is more marked (Fig. 8).

*Relaxation.*—The relaxation of dead muscle when heated to 50–60° C. is active (Singh and Singh, 1954 *a*, *b*, *c*). But if the muscle is heated to

65–70° C., then the relaxation of the thermal contraction is passive. By active relaxation of dead muscle is implied that it relaxes without the application of an external force.

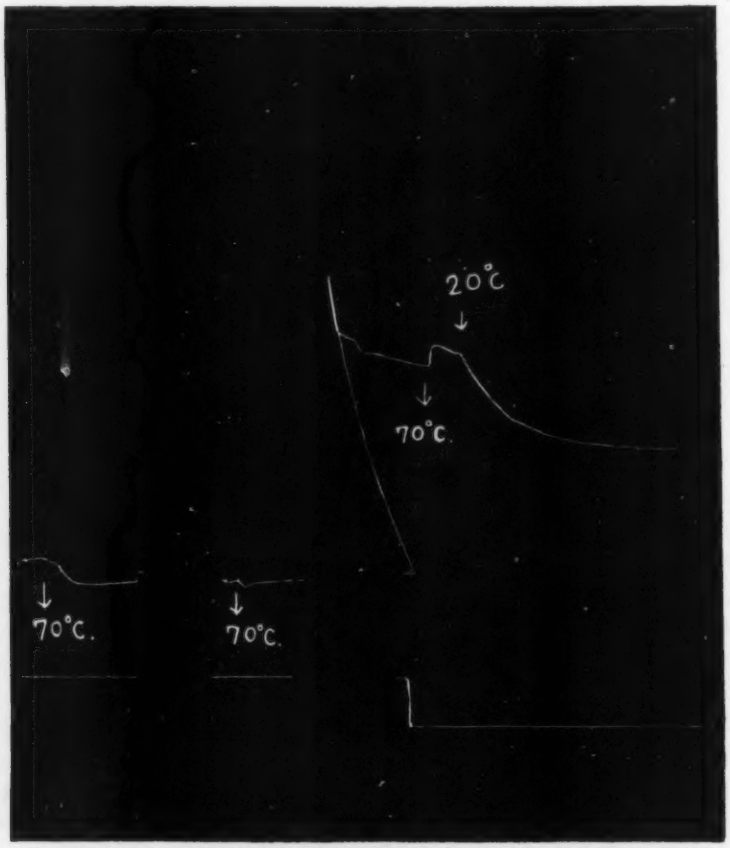


FIG. 8. Dog's sromach muscle killed by heating to 50° C. The muscle is not very sensitive; so the initial length is increased. The initial tension now increases. The thermal response has now increased. But the muscle relaxes much beyond the starting point.

It has been shown above that if the muscle has been previously heated to 50° C. then heating to 50–60° C. produces only relaxation or a feeble contraction. After one of the relaxing mechanisms has been inactivated by heating to 70° C., the thermal response increases. This again suggests that the normal response of unstriated muscle is the net result of two opposing forces. One produces relaxation and the other contraction; if the former

is inactivated, then the contraction is increased. The muscle may thus be contracting and relaxing simultaneously. The relaxing mechanism may also be inactivated by increase in initial length; this is probably one of the causes which produces increasing tension with increase in initial length. The effect of increase in initial length in antagonising inhibition, is well shown in living muscle.

## Discussion

These experiments show that the contractile system of unstriated muscle consists of two components; in one the relaxation is active and in the other passive. The former is inactivated by heating to 70° C. Further the contraction of dead muscle, in which the relaxation is passive, resembles the twitch contraction. These experiments, therefore, substantiate the views mentioned previously that unstriated system consists of two systems; in one the relaxation is active and in the other, passive (Singh and Singh, 1950, 1951 *a*; 1952; Singh, 1953).

The system in which the relaxation is passive, consists of two components; one component is inactivated by heating to 70° C., as the relaxation becomes very slow. Striated muscle contains predominantly this component, and this may therefore be termed as striated muscle component (SMC). In some unstriated muscles, there is a component relaxing passively, which is not inactivated by heating to 70° C.; this may be considered as a pure unstriated muscle component (UMC). Cardiac muscle and some unstriated muscles are of intermediate variety. It is interesting to note that histologically also, cardiac muscle is intermediate between striated and unstriated muscles. There is thus a correlation between histological appearance and the response of the contractile mechanism of muscle. Muscle therefore appears to be made of two structural components (SMC and UMC), which relax passively, in addition to the component which relaxes actively (ARC, active relaxation component). Different muscles differ in having these in different proportions.

The properties of the thermal contraction show that the staircase effects and fatigue phenomena are at least partially due to the contractile mechanism, though the excitatory system may also be involved in living muscle. Contractions produced by heat and by hydrochloric acid show that the increasing tension produced by increase in the initial length, is due to the contractile mechanism.

These experiments also show that the contractile mechanism of unstriated muscle is a variable entity; this was also shown by contraction of dead muscle produced by hydrochloric acid.

## SUMMARY

1. The properties of the contractile mechanism of unstriated muscle have been studied by recording the contraction of heat killed muscle, produced by raising its temperature to 70° C.

2. The effects of heat show that the contractile mechanism of unstriated muscle consists of two components; in one the relaxation is active and in the other passive. The latter again consists of two parts one of which is activated by heat.

3. The thermal contraction of heat killed unstriated muscle resembles the phasic response of living muscle.

4. The thermal response of heat killed unstriated muscle shows staircase and fatigue effects. It increases with initial length up to a certain point, so that there is an optimum length of muscle for its production. These phenomena in living muscle are therefore properties of the contractile mechanism.

5. Starling's law of the heart is also shown by thermal contraction of dead muscle.


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# FURTHER OBSERVATIONS ON THE RELATION BETWEEN DENATURATION OF PROTEINS AND RELAXATION OF UNSTRIATED MUSCLE

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Received January 27, 1955

According to modern theory, the contraction of muscle is presumed to be due to folding of the polypeptide chains of the contractile muscle proteins, but so far no unequivocal evidence of chain folding in the contracted state of muscle has been produced. Such a folding, however, has been demonstrated by an indirect method; it has been shown that substances which denature proteins, cause active relaxation of the contractile mechanism of unstriated muscle (Singh and Singh, 1954 *a*, *b*, *c*). As denaturation is supposed to be due to the unfolding of the polypeptide chains, this suggests that relaxation is due to unfolding of such chains and contraction to an opposite process; experiments on *Mytilus* muscle suggest that contraction is due to a globular transformation of the contractile proteins (Singh and Singh, 1951).

In the present research, the effect of some other substances which are known to denature proteins, has been further studied on the contractile mechanism of unstriated muscle; previous results have been substantiated.

## Experimental

These experiments were performed on unloaded strips of circular muscle of the stomach of the frog, *Rana tigrina*. Both dying muscles and heat killed muscles were used. The muscles were killed by raising the temperature of the saline from room temperature to 50° C. in about 5 minutes and keeping at that temperature for 1 minute. The dying and heat killed muscles were immersed in various experimental solutions for 24 hours, unless otherwise stated (Singh and Singh, 1949, 1951, 1954 *a*, *b*, *c*).

Various substances were dissolved in frog saline and their effect on the contractile mechanism was noted. Alcohols, formalin and phenol were dissolved in frog saline at pH 8 (borate buffer), and copper sulphate and mercuric chloride in unbuffered saline.

## RESULTS

*Effect of Alcohols.*—Some alcohols are known to denature and coagulate proteins, so the effect of these was tested on the contractile mechanism. It has been shown previously, that when the muscle is immersed in distilled water, small concentrations of methyl, ethyl, propyl and butyl alcohols, increase the effect of distilled water in causing active relaxation, and larger concentrations have the opposite effect (Singh and Singh, 1949). The same effect is observed if these alcohols are dissolved in saline; heated and dying muscles behave similarly.

Ethyl alcohol, in concentrations of 5 to 20 per cent., causes active relaxation of dying and heated muscle by about 20 to 45 per cent. (Figs. 1, 2). Methyl alcohol has a similar effect. 5 per cent. propyl alcohol causes similar active relaxation by 40–50 per cent.; 10 per cent. produces lengthening by about 20–30 per cent.; 15 per cent. produces active relaxation by about 10–15 per cent.; 20 per cent. produces no effect or slight contraction.

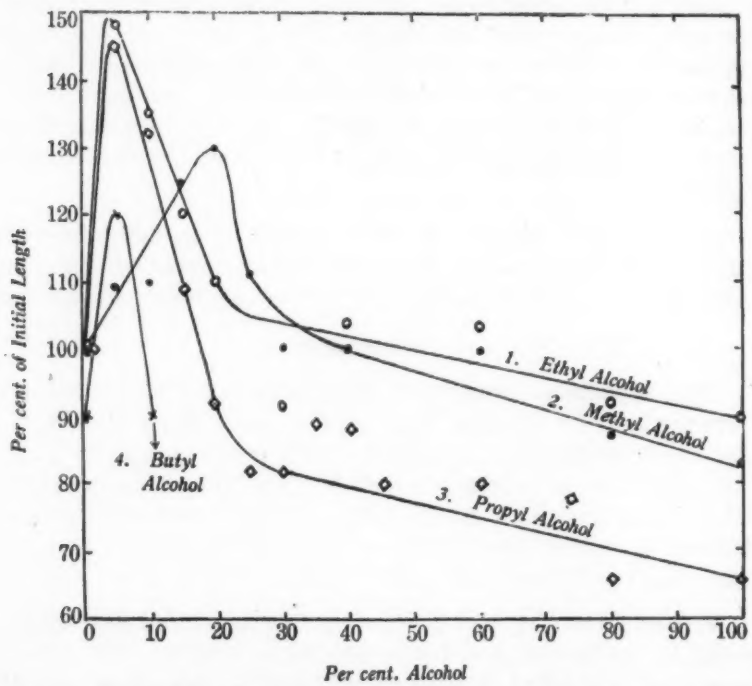


FIG. 1. Dying frog's stomach muscle. Effect of ethyl, methyl, propyl and butyl alcohols on length of unloaded muscle. Initial length is the length in saline. Immersed for 24 hours.

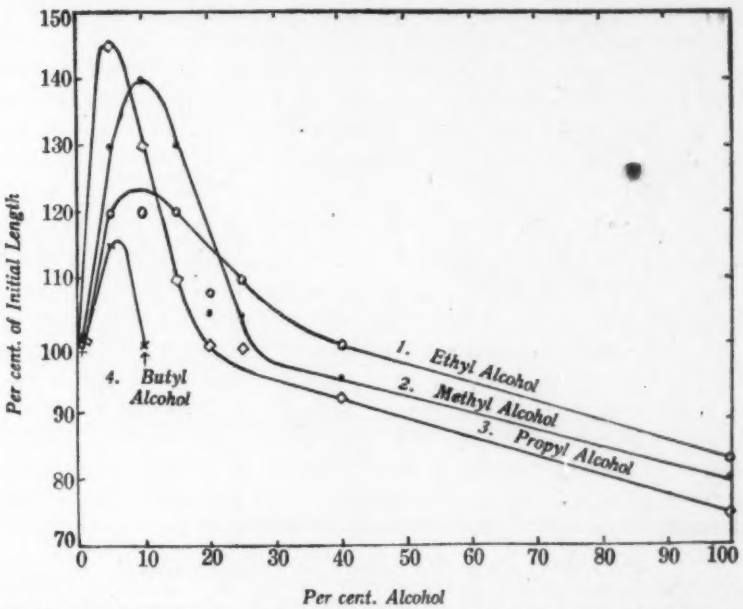


FIG. 2. Heated frog's stomach muscle. Heated to 50° C. for one minute. Effect of ethyl, methyl, propyl and butyl alcohols on length of unloaded muscle. Initial length in saline. Immersed for 24 hours.

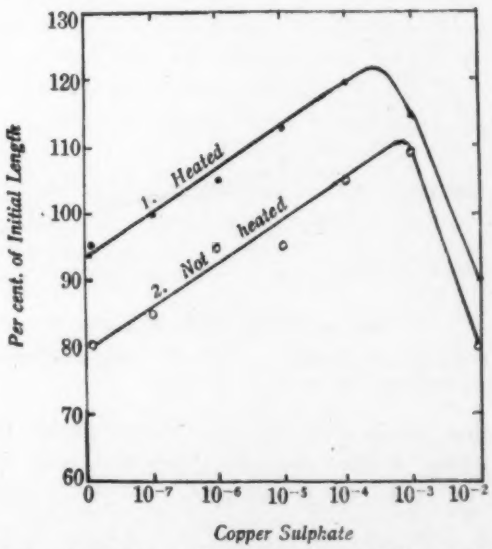


FIG. 3. Frogs' stomach muscle. I Heated muscle. II Dying muscle. Effect of copper sulphate on length of unloaded muscle. Immersed for 24 hours.

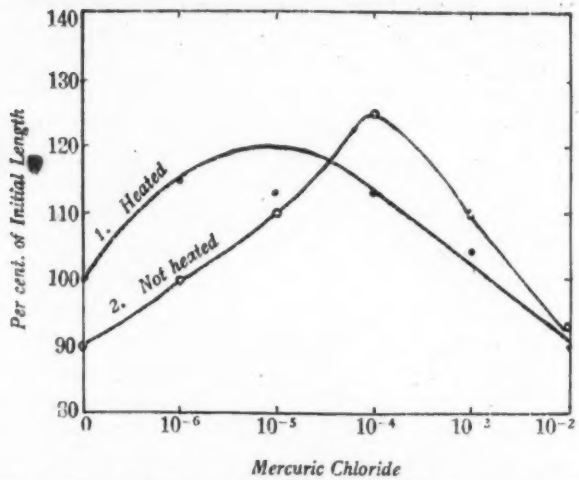


FIG. 4. Frog's stomach muscle. I Heated muscle. II Dying muscle. Effect of mercuric chloride on length of unloaded muscle. Immersed for 24 hours.

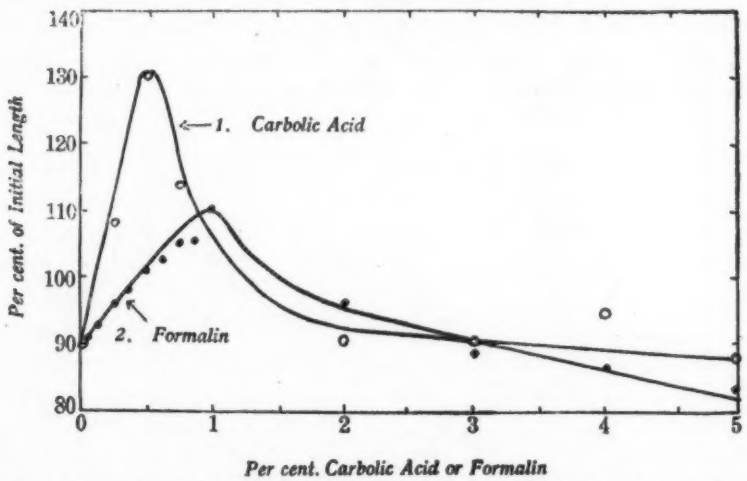


FIG. 5. Frog's stomach muscle. Effect of carbolic acid and formalin on length of unloaded dying muscle. Immersed for 24 hours.

Higher concentrations produce contraction. Propyl alcohol produces greater contraction than methyl or ethyl alcohols. 5 per cent. butyl alcohol produces active relaxation by about 10–20 per cent.; saturated solution produces contraction.

*Effect of Metallic Salts.*—Salts of heavy metals are known to denaturate proteins. The effect of copper sulphate and mercuric chloride was tested. Copper sulphate in small concentrations, 1 in 1,000, produces active relaxation by about 30 per cent.; 1 per cent. produces contraction (Fig. 3). Mercuric chloride has similar effect; 1 in 10,000 produces active relaxation by about 40 per cent. and higher concentrations, 1 per cent. produces contraction (Fig. 4).

*Effect of Other Substances.*—Carbolic acid and formalin produce active relaxation in small concentrations and contraction in higher concentration. Formalin, 1 per cent. produces active relaxation by about 10 per cent. and carbolic acid, 0·5 per cent. by about 30–40 per cent. (Fig. 5). These substances produce contraction in strength of 5 per cent.

## Discussion

A number of dissimilar substances have been shown to produce active relaxation of the contractile mechanism of unstriated muscle. The common known effect of these substances is denaturation of proteins in small concentrations, and coagulation in higher concentrations. These experiments support the idea that denaturation of proteins is accompanied by unfolding of polypeptide chains. Relaxation of muscle, therefore, appears to be similar to denaturation. Contraction appears to be similar to coagulation of proteins (Singh and Singh, 1954 *a*, *b*, *c*; 1955).

## Summary

1. Small concentrations of methyl, ethyl, propyl and butyl alcohols cause active relaxation of the contractile mechanism of unstriated muscle; larger concentrations produce contraction.

2. Copper sulphate, mercuric chloride, carbolic acid and formalin have similar effects.

3. The above substances are known to produce denaturation of proteins in small concentrations and coagulation in higher concentrations; it therefore appears that active relaxation is akin to denaturation and contraction to coagulation of proteins.

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468-55 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao Superintendent.
and Published by The Indian Academy of Sciences, Bangalore.

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